substitution (GTA valine, ATA isoleucine) as well as the fourth SNP (rs1764391: CCC proline, TCCserine). For rs41266431 patients with homozygosity for the G variant (n=11) had higher IL-8 levels (median: 12.3/6.7 pg/ml, p<0.11) in whole blood than those showing heterozygosity for the G/A mutation (n=7). **Conclusion:** More patients have to be analyzed to substantiate these findings. For GJA4 the SNP at rs 41266431 seems a promising candidate gene.

P1441

One size does not fit all – Impact of the one liter tidal volume breathing protocol on indices from nitrogen multiple-breath washout in children Sophie Yammine, Florian Singer, Philipp Latzin. Department of Paediatric Pneumology, University Children's Hospital of Bern, Switzerland

Background: Nitrogen multiple-breath washout (N2MBW) is a useful tidal breathing lung function test to assess ventilation inhomogeneity (VI), e.g. in Cystic Fibrosis (CF) patients. One liter tidal volume (VT) breathing protocols are regarded to improve comparability of results between subjects breathing at different VT and are widely used in adults. The impact of protocols using fixed VT on results in children is unknown. We assessed whether breathing at fixed VT impacts N2MBW indices in school-aged children.

Methods: Ten children with CF and 16 healthy children performed six N2MBW tests using a validated setup (Exhalyzer D, Eco Medics, Switzerland). Children performed three baseline N2MBW at free tidal breathing and three N2MBW at increased VT with a target of one liter using an incentive. Outcomes were size and variability of lung clearance index (LCI), functional residual capacity (FRC), Scond and Sacin.

Results: All 26 children achieved six N2MBW. Mean (SD) VT at free tidal breathing was 0.5 (0.1) L, at fixed VT 1.2 (0.2) L. Comparing free tidal breathing with the one liter VT N2MBW, LCI increased on average (95% CI) 2.3 (0.6-3.9) in CF children and 1.4 (0.5-2.3) in healthy children. LCI increased by more than one LCI unit in 9 out of 10 CF children and in 14 out of 16 healthy children. FRC decreased, Scond and Sacin increased significantly. Variability within tests and between subjects increased for all MBW outcomes.

Conclusion: Fixed tidal breathing protocols impact N2MBW indices as shown for the one liter VT protocol in school-aged children. Underlying physiological mechanisms and applicability in adult patients require further study.

P1442

Epithelial cell regulation of immunity in cystic fibrosis

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Introduction: Despite clinical importance, the adaptive immune system in cystic fibrosis (CF) lung disease has been sparsely studied.

Methods: We isolated CF primary human bronchial epithelial cells (PBEC) and assessed their modulation of monocyte-derived dendritic cell (moDC) function and downstream T cell activation, hypothesising that epithelial cells skew immunity to favour chronic infection and lung damage in CF. Healthy monocytes were cultured with conditioned medium from 6 steady-state CF patient PBEC during moDC differentiation with IL-4 and GM-CSF.

Results: Compared to control moDC, epithelial cell conditioned moDC were tolerogenic and macrophage-like (\downarrow CD1a, [darr] CD86, \uparrow CD14 and \uparrow IL-10), inducing low T cell proliferation and interferon- γ production in an allogeneic mixed



Figure 1. Steady state moDC phenotype and function altered by PBEC derived soluble factors (MFI - median fluorescence intensity, CPM - counts per minute).

154. Cystic fibrosis (adults and children): new basic and clinical physiology research

P1440

Connexin 37 and Connexin 43 genotypes in correlation to cytokines in induced sputum and blood in cystic fibrosis (CF) $\,$

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Background: We have provided evidence in previous studies that cytokines (IL-8, TNF alpha, LBP) measured in whole blood correlate negatively with lung function in delta 508 homozygous patients. GAP junction proteins (connexins) might be of importance for the influx of blood cells into the lung. Our aim was to assess the relationship between connexin genotypes, cytokines (IL-8, TNF-alpha and LBP) in induced sputum and blood and lung disease.

Methods: 24 patients homozygous for delta F 508 (median age 20.5 y, m/f 14/10, BMI 20,35 kg/m², Shwachman score 75, FEV₁(%) 83) were examined. Sequence analysis was performed for GAP junction protein alpha 1 (GJA1/Connexin 43) and gap junction protein alpha 4 (GJA4/connexin 37). Cytokines were assessed in blood and induced sputum (IS) by chemiluminescence (DPC Biermann, Bad Homburg, Germany).

Results: Here we present the first preliminary data: For 18 patients cytokine and sequence data were available. Whereas GJA1 showed only one rare heterozygous SNP (rs138386744) in one patient, four common SNPs were detected in GJA4. Two were synonymous changes, but the third variant (rs41266431) causes an amino acid

lymphocyte reaction (MLR). Stimulation of PBEC or direct stimulation of moDC with clinically isolated *Burkholderia cenocepacia* whole cell lysate gave a mature, highly stimulatory moDC phenotype while *Pseudomonas aeruginosa* induced poor maturation and a less potent T cell response.

Conclusion: CF epithelial cells secrete factors which contribute to immune tolerance. CF pathogens may have a variable ability to overcome this regulation and induce an immune response which may favour chronic infection by *Pseudomonas aeruginosa*.

P1443

Proteases from anaerobic bacteria cleave naturally occurring innate antiproteases in cystic fibrosis

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Introduction: Cystic fibrosis (CF) results in chronic bacterial pulmonary infection leading to an irreversible decline in lung function and ultimately respiratory failure. There is evidence now that CF infection is polymicrobial and that anaerobic bacteria which are not usually detected by routine culture are responsible for at least in part for pathogenic infection (1).

Aim: We aim to study if proteases from *P. melaninogenica*, the most common anaerobic bacteria found in the CF lung (2), cleave the host innate human antiproteases namely, alpha one antitrypsin (AAT), secretory leukocyte protease inhibitor (SLPI) and elafin.

Method: *P. melaninogenica* is grown and incubated at 37°C in Luria Bertani Broth (LB) broth, and Basal Anaerobic Media (BAM) broth under strict anaerobic conditions in an anaerobic cabinet. Recombinant SLPI, Elafin and native AAT were incubated for selected time points with supernatant and cleavage products visualised by SDS-PAGE electrophoresis and Western Blotting analysis using specific antibodies raised against these antiproteases.

Results: *P* Melaninogenica produces proteases on Day 4,5,6 of incubation and they have distinct cleavage patterns and these cleavage products cleave naturally occurring antiproteases.

References:

- Worlitzsch, D., Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. J. Clin. Invest., 2002. 109: p. 317-325.
- [2] Tunney, M.M., Field, T.R., Moriarty, T.F., Patrick, S., Doering, G., Muhlebach, M.S., Wolfgang, M.C., Boucher, R., Gilpin, D.F., McDowell, A., Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. Am. J. Respir. Crit. Care Med, 2008. 177: p. 995-1001.

P1444

Pseudomonas aeruginosa counteracts the host defense functions of MIG/CXCL9

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Pseudomonas aeruginosa is a frequent colonizer and cause of exacerbations in cystic fibrosis (CF) and other chronic pulmonary diseases. CF is a monogenic disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. One feature of the disease is a reduction or absence of chloride transport and dysregulation of sodium transport to the airway epithelium surface. In this study, we show that the antibacterial chemokine MIG/CXCL9 (monokine induced by IFN- γ) is expressed by bronchial epithelial cells and could be detected in sputum from patients suffering from CF. MIG/CXCL9 showed high bactericidal activity against P. aeruginosa in vitro, also at salt concentrations similar to those seen in CF. P. aeruginosa strains equipped with efflux-pumps, showed a high degree of resistance to MIG/CXCL9. Elastase from P. aeruginosa and neutrophils respectively, both cleaved of a distinct fragment from the MIG/CXCL9 holoprotein. Taken together, our data indicates that the activity of antimicrobial proteins, as exemplified by MIG/CXCL9, is not foremost hampered by salt but rather efflux-pumps and proteases. The latter released both by the bacteria and neutrophils

P1445

Sex-dependent transmission ratio distortion in a SNP in calreticulin (CRT) in CF patients

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Objectives: A difficulty in CF is the great phenotypic variability. Genes interacting with CFTR might be potential modifiers of CF lung disease. Thus, we are assessing the impact of SNPs of interacters on CF disease outcome.

Methods: 78 SNPs were genotyped in 93 well-characterized F508del homozygous CF patients. Crt6, a SNP in CRT, was additionally genotyped in 151 healthy controls. Pearson's chi-squared test was used to test for deviations from Hardy-

Weinberg Equilibrium (HWE). SNPs were correlated to repeated measurements of lung function parameters by applying linear-mixed-effect model analysis. **Results:** We genotyped 78 SNPs in 10 genes. Of these SNPs 42 showed an allele

Results: We genotyped 78 SNPs in 10 genes. Of these SNPs 4.2 showed an affele frequency <10% and thus were excluded. We found 6 SNPs in 4 genes to be significantly associated with at least one lung function parameter (paper in progress). Interestingly, all SNPs were in HWE except Crt6. The critical value for the χ^2 is 3.84 reflecting p=0.05 and therefore a significant difference between observed and calculated genotypes. Opposed to the male population with a χ^2 of 0.49 the observed genotype distribution in the female CF population is significantly different from the expected distribution with a χ^2 of 10.22 (p~0.001). In the control group the male as well as the female population are in HWE.

Conclusion: Our findings indicate that there is a sex-dependent effect in Crt6 resulting in a shift of the wildtype to the heterozygous genotype in the female population. This effect did not reproduce in the control population. Either this effect is specific for CF, or it is due to the small population size. We are currently testing our hypothesis in a second independent CF cohort.

P1446

Endoplasmic reticulum stress in circulating cystic fibrosis neutrophils <u>Killian Hurley</u>, William Leitch, Nessa Banville, Stephen Cox, Kerstin Pohl,

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Introduction: Cystic fibrosis (CF) is caused by a defect in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. A number of studies have been performed to investigate whether neutrophil dysfunction in CF is primarily as a result of the genetic defect or due to chronic bacterial infection and inflammation. **Aim:** The aim of this study was to provide support for intrinsic alterations in CF, specifically to determine whether accumulation of CFTR within the endoplasmic reticulum (ER) of circulating neutrophils from patients with CF leads to ER stress responses including the release of ER calcium (Ca²⁺) stores. This study focused on the ER-resident chaperone, GRP78 and ATF6, a transcription factor that coordinates the unfolded protein response (UPR).

Methods: Neutrophils were purified from whole blood and the cytosols were analysed using Western blotting for ER stress markers ATF6 and GRP78. Intracellular Ca²⁺ was determined using a fluorometric assay.

Results: Western blots revealed that markers of ER stress, GRP78 and cleaved ATF6, were increased in neutrophil cytosols of patients with CF homozygous for the Δ F508 mutation, when compared to healthy controls (n=8 for both groups). In addition, densitometric analysis of immuno-bands confirmed significant upregulation of both GRP78 and active ATF6 in CF neutrophils compared to control cells (p<0.05). Intracellular Ca²⁺ was increased in the CF neutrophils compared to healthy controls.

Conclusions: Our data demonstrates for the first time activation of the UPR *in vivo* in neutrophils isolated from individuals with CF, which may in part explain the exaggerated inflammatory response of these cells.

P1447

Cytokine and chemokine release in response to *Pseudomonas aeruginosa* (PA), by bronchial epithelium of the native airway and transplanted lung of paediatric cystic fibrosis (CF) lung transplant recipients

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Introduction: Infection and inflammation are implicated in the pathophysiology of Bronchiolitis Obliterans Syndrome (BOS), the major cause of mortality following lung transplantation. It is unclear if the cytokine and chemokine release by Cystic Fibrosis (CF) airway epithelium in response to pathogens differs from that of the transplanted lung.

Aims: We hypothesised that there is no difference in the cytokine and chemokine release in response to *Pseudomonas aeruginosa* (PA), by the epithelium of the native CF airway and the transplanted lung.

Methods: 5 children who had lung transplantations for CF (Great Ormond Street Hospital for Children, London, UK), were studied. Bronchoscopic brushings from above and below the airway anastomosis were cultured to differentiated ciliated epithelium in an air-liquid interface (ALI). The epithelium was exposed to late exponential cultures of PA (10⁶ CFU per ml). The culture supernatants were harvested at baseline and 5 hours post PA exposure. The cytokines and chemokines in the culture supernatants were measured using a multiplex ELISA based protein array (SECTOR Imager 6000, MSD).

Results: There were no differences in baseline levels of cytokines and chemokines. 5 hours after exposure to PA, the release of chemokines - CCL2, CCL5, CCL13, CXCL8 and the cytokines - IL1 β , IL13 and TNF α , by the native CF epithelium was significantly higher (p<0.01) compared to that from the transplanted lung. **Conclusion:** The differential cytokine and chemokine release in response to pathogens may be contributory to the exaggerated inflammatory response of the CF epithelium.

P1448

Ciliary function of the nasal and bronchial epithelium in children with cystic fibrosis

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Background: Normal ciliary function is essential in pulmonary defence. It is unclear if there is any difference in ciliary function between the nasal and the bronchial epithelium, in children with Cystic Fibrosis (CF).

Aims: Our aim was to determine if there is any difference in ciliary function between the nasal and the bronchial epithelium, in children with CF.

Methods: 9 children with CF (2 males, median [IQR] age: 14.1 [12.8-15.7] years, median [IQR] duration post transplant: 12 [2.9-15.5] months), who had lung transplantation at the GOS Hospital for Children (London, UK) were studied. Nasal brushings and bronchoscopic bronchial brushings from the lower airway above the anastomosis were studied using digital high speed video camera to determine the ciliary beat frequency (CBF) and beat pattern, as described previously. (Thomas B. et al. Eur Respir J. 2009;34:401-4).

Results: There was no significant difference in CBF or beat pattern between the epithelium of the nose and the lower airway, in children with CF (Table 1). However, the CF nasal epithelium showed significantly lower CBF and higher dyskinesia index compared to that of healthy children (Data published previously, Table 1)

Table 1

	CF Bronchial epithelium	CF Nasal epithelium	Healthy nasal epithelium †
CBF (Hz) Dyskinesia index (% Immotility index (%	7.4 [6.4-9.2] (42.2 [32.6-56.4] (4.1 [3.0-5.9]	9.5 [7.5-10.8]* 55.4 [45.8-62.0]* 0 [0-11.4]	13.4 [11.6-14.2] [†] 0 [0-25] [†]

Data expressed as median [IQR]. *p<0.01 compared to data on healthy nasal epithelium. [†]Thomas B, et al. Eur Respir J 2009; 34:401-4.

Conclusion: There was no difference in ciliary function between the nasal and the bronchial epithelium in paediatric CF lung transplant recipients.

P1449

Detection of antibodies against Pseudomonas aeruginosa in the sputum of cystic fibrosis patients: A pilot study

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Chronic Pseudomonas aeruginosa (Pa) infection plays a pivotal role in disease progression in patients with cystic fibrosis (CF). The aim of this cross-sectional study was to test whether anti-Pa antibodies can be detected in the sputum using common serological methods.

During routine ambulatory visits blood and spontaneously expectorated sputum samples were obtained from 29 adult CF patients (11 chronically infected with Pa [Pa+], 10 non-infected [Pa-] and 8 intermittently infected [Pa+/-]). Anti-Pa antibodies (IgA and IgG) in serum and in sputum supernatant were measured with ELISA originally developed for measuring anti-Pa antibodies in serum (Genesis Diagnostics). Data are presented as means±SEM.

As expected serum IgA levels were elevated in Pa+ as compared to Pa- patients $(20.3\pm9.5 \text{ vs. } 6.0\pm1.5 \text{ pg/ml}, \text{ respectively, } p<0.05)$. Sputum IgA levels were above the detection limit of the assay in all subjects, and were significantly increased in Pa+ compared to Pa- patients (20.6±5.6 vs. 8.5±1.1 pg/ml, respectively, p<0.05). In Pa+/- patients sputum IgA levels were similar to that of Pa- patients (p>0.05). In all subjects serum and sputum IgA levels showed a close correlation (r=0,525, p<0.01). The sensitivity and the specificity of the assay in sputum were 70 and 72%, respectively, as compared to 44 and 90% in serum. Sputum IgG antibody concentrations remained under the detection limit of the assay in most cases

In conclusion, sputum anti-Pa IgA antibody levels are elevated in patients with chronic Pa infection. The sensitivity of the assay is even greater in sputum than in serum raising the possibility that sputum could be material of choice for the early detection of Pa.

P1450

Tracking disease progression in cystic fibrosis using bronchiectasis, trapped air and quality of life

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Background: Progression of cystic fibrosis (CF) is characterized by bronchiectasis (BE) and trapped air (TA) on CT. We hypothesize that progression of BE, and TA results in a lower Health Related Quality of Life (HRQoL), assessed by the Cystic Fibrosis Ouestionnaire-Revised (CFO-R).

Objective: To evaluate associations between changes in BE, TA, and CFQ-R over time

Methods: Cohort study (July 2007-January 2012). Clinical stable CF patients, with two routine bi-annual chest CTs, and CFO-Rs. CT scans were anonymous and randomly scored, using CF-CT BE and TA scores. Scores are expressed as % of maximum score. CFQ-R was completed by children (aged 6-13 years) and adolescents (aged ≥ 14 years). Score-range 0-100, higher scores indicate better HRQoL. For changes in CF-CT BE, TA score, CFQ-R respiratory-symptoms domain scores, their correlations, and to test the hypothesis we used Student's paired t-test, Spearman's correlation coefficient, and linear regression model, adjusted for age and gender.

Results: CF patients (n= 40): mean age T1 11.9 years, T2 13.8 years. In two years there was a significant increase in CF-CT BE scores (p=0.03) and CF-CT TA scores (p=0.03), but not in CFQ-R scores. At T1 CF-CT BE scores (p<0.01, r=-0.49) and CF-CT TA scores (p=0.04, r=-0.34) correlate with CFQ-R scores. At T2 similar correlations were found: CF-CT BE (p<0.01, r=-0.41), CF-CT TA (p=0.02, r=-0.37). Change in CF-CT BE and TA scores did not correlate to change in CFO-R scores

Conclusion: BE and TA correlates to HRQoL. Modest progression of BE and TA did not result in impairment of HRQoL

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P1451

Lung clearance index (LCI) and hyperinflation in children with cystic fibrosis (CF)

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Introduction: LCI is used to detect early CF lung disease.^{1,2,3} However, there are few data relating changes in LCI to lung volumes, and extent of hyperinflation, in children with CF^1 Therefore, we aimed to look at the association between LCI derived from multiple-breath nitrogen washout (MBNW) and two indices of hyperinflation (i) residual volume/total lung capacity (RV/TLC), and (ii) the difference between FRC determined by plethysmography (FRCp) and MBNW (FRC_{N2})

Methods: Children with CF completed MBNW and plethysmography as part of their annual review. All tests were performed, analysed and reported according to ATS/ERS recommendations. The difference between FRC_p and FRC_{N2} was expressed as a % of FRC_p and called $FRC_{\rm diff}.$ Results were examined using Spearman's rank correlation coefficient.

Results: 37 children with CF (aged 5-17) completed lung function tests. A significant correlation was found between LCI and RV/TLC (r=0.516, p=0.001) (Figure), and between LCI and FRCdiff (r=0.413, p=0.011).



Discussion: The adoption of LCI as an ideal marker for early lung disease requires that it should correspond to established markers of abnormalities in lung function. Our findings showed comparable decline in LCI and indices of hyperinflation in children with CF. **References:**

[1] Horsley, A. et al. 2008; Respir Physiol and Neurobiology; 162: 197-203

[2] Aurora, P. 2010; Thorax; 65: 373-374

[3] Gustafsson et al. 2008; Thorax 2008 63: 129-134.

P1452

Short term effects of chest physiotherapy in children with cystic fibrosis assessed by a new lung function test

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Background: No lung function test exists that is able to assess short-term effects of physiotherapeutic treatment (PT) in children with Cystic Fibrosis (CF). We recently developed a tidal single-breath washout (SBW) using two tracer gases to measure ventilation inhomogeneity (VI).

Aims: We assessed whether this new SBW test is able to measure short-term effects of PT and inhalation.

Methods: Children with CF (n=25) between 6 and 16 years performed lungfunction assessments prior to and after inhalation and PT. Assessments consisted of a double tracer gas SBW (DTG-SBW) and spirometry. DTG contained sulfur hexafluoride (SF6) and helium (He), and was inhaled during tidal breathing. A side-stream ultrasonic flowmeter measured molar mass. DTG-SBW outcome was percentage of expired volume where expired molar mass equals inspired molar mass, reflecting inspired ratio of SF6 and He (IPDTG).

Results: After intervention IPDTG decreased from 66.5% (± 25.8) to 59.7% (± 25.4) resulting in a mean difference of -7.3% (95%CI -12.9 to -1.8) and MEF25-75 increased from 1.41 L/s to 1.62 L/s resulting in a mean difference of 0.19 L/s (95%CI 0.02 to 0.37). In a post-hoc subgroup analysis we found that the DTG-SBW is more suited to detect changes in VI in patients with mild CF lung disease (n=14, FEV1 z-score >-2), whereas spirometry indexes increased only in patients with moderate CF lung disease (n=11, FEV1 z-score <-2).

Conclusion: The DTG-SBW seems to be a promising test to detect short-term effects of physiotherapy and inhalation.

P1453

Peripheral airway function and severity of CF lung disease – A crosssectional study from childhood to late middle age

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Background: Ventilation inhomogeneity (VI) measured by multiple-breath washout (MBW) is a characteristic feature of cystic fibrosis (CF) and correlates more closely than spirometry with HRCT structural lung damage (Thorax 2008;63:129). MBW provides both global indices of VI such as the lung clearance index (LCI) and specific indices of VI arising in the conductive (Scond) and the acinar (Sacin) airway zones (Respiration 2009;78:339). It is unknown how Scond and Sacin reflect overall lung disease severity over a wide age range.

Methods: The LCI, Scond and Sacin were measured by SF_6 MBW in 71 CF subjects aged 7.1-55.4 yrs (median 17.4) with FEV₁ % pred between 19 and 127% pred (median 86). Data are given as z-scores (Respiration 2009;78:339).

Results: FEV₁ was abnormal (z-score < -1.96) in 27 (38%), LCI in 57 (80%), Scond in 67 (94%) and Sacin in 40 (56%) of subjects. Correlations between indices are given in Table 1. MBW indices were more sensitive to airway pathology than spirometry. Scond was the most sensitive index but did not correlate with disease severity. LCI reflected spirometric disease severity better than Sacin or Scond and was more sensitive than Sacin.

Table 1. Table of correlation between lung function indices

Dependent	VS	Independent	R2	p-value
LCI	vs	FEV1	0.64	< 0.001
Sacin	vs	FEV1	0.47	< 0.001
Scond	vs	FEV1	0.00	n.s.
Sacin	vs	LCI	0.75	< 0.001
Scond	vs	LCI	0.00	n.s.

Conclusion: These cross-sectional findings suggest that LCI is more useful than Scond or Sacin in monitoring CF lung disease. Scond may, however, be used as an early marker of lung involvement.

P1454

Prolonged oxygen kinetics during submaximal cardiopulmonary exercise testing (CPET) in adult patients with cystic fibrosis (CF) and their relationship with indices of oxidative capacity

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Background: Patients with cystic fibrosis demonstrate reduced exercise capacity. As the submaximal exercise testing overcomes many of the limitations of maximal exercise testing, indices of submaximal exercise capacity like time constant (t_{on}) are of increased investigational interest.

Aim: To compare oxygen kinetics of submaximal exercise testing between patients

with cystic fibrosis and healthy controls and explore their relationship with indices of oxidative capacity.

Patients and Methods: 14 adult patients with CF (8 males/6 females, mean age 22) and 10 healthy controls (5 males/5 females, mean age 29) submitted to maximal and submaximal CPET. We have calculated: ton during submaximal CPET, peak oxygen consumption (VO2peak) during maximal CPET, anaerobic threshold (AT) as a percentage of VO2peak and the slope of recovery after maximal CPET as it is expressed by the ratio of oxygen consumption to time (VO2t slope).

Results: The main finding is that t_{on} in control group is significantly lower (29.35±6.38 s) than t_{on} in CF group (42.35±21.55 s, p<0.05). Moreover, a statistically significant negative correlation is found between t_{on} and indices of oxidative capacity of maximal CPET such as: VO2peak (r= -0.533, p=0.049), AT % (r= -0.645, p=0.013) and VO2/t slope (r= -0.576, p=0.031).

Conclusion: Oxygen kinetics of submaximal CPET in adult patients with cystic fibrosis are prolonged and they are correlated with indices of oxidative capacity during maximal CPET.

P1455

Habitual physical activity in cystic fibrosis

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Introduction: Habitual physical activity has important clinical implications in Cystic Fibrosis (CF) and has therapeutic effects on sputum clearance, respiratory muscle strength and quality of life. While recent studies have shown that aerobic fitness is related to survival, that physically active CF patients have higher peak oxygen uptake (VO2peak) and lower rate of decline of FEV1, less is known about the relationship between physical activity levels and patients's functional status, including exercise tolerance.

Methods: Eleven stable CF patients (mean (SD) age 32 (9) yrs; FEV1 2.7 (0.8) l; IC 3.8 (0.9) l), were studied at rest, during symptom-limited incremental exercise test (CPET) and during 6MWT. We assessed daily physical activity using both SW (SenseWear, SW) that subjects wore for 4 consecutive days from waking until going to bed including weekends, and the Habitual Activity Estimation Scale Questionnaire (HAESQ).

Results: There was no difference between the weekdays and weekends recordings in any activity variable. We found no agreement in physical activity measured by SW vs HAESQ. By contrast, we found a close relationship between SW step count and FEV1 (r2=0.9; p<0.02) and SW energy expenditure vs VO2peak (r2=0.8; p<0.05) at CPET. Distance at 6MWT was not correlated to any physical activity variables, either at SW or HAESQ.

Conclusions: Adults CF patients have similar habitual physical activity levels at weekdays and weekends. Activity levels measured by the subjective methods and by the objective monitoring, i.e., SW vs HAESQ, seems to provide different information on the level of daily physical activity. Physical activity is related to degree of airflow obstruction and to the maximum exercise capacity.

P1456

Acute exercise in cystic fibrosis patients increases neutrophilic pulmonary inflammation

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Background: Neutrophilic inflammation mainly from recurrent bacterial infection characterizes cystic fibrosis (CF) airways. Repeated inflammatory insults may lead to progressive lung function decline. Acute exercise is reported to trigger a systemic inflammatory response, but inflammation pattern has been poorly studied. As exercise is part of a healthy lifestyle, we aim to depict the modulation of inflammation after an acute exercise in CF.

Methods: 12 subjects with mild to moderate stable CF (FEV1>50% of p.v.) underwent a constant load exercise test at 80% of their maximal load. Sputum and blood were sampled before and 1 hour after exercise and analysed for differential leucocyte counts.

Results: 6 females and 6 males completed the study with a mean FEV1 of 72.2% and a mean exercice time of 4.6 minutes. No desaturation was reported. Table 1 summarizes the differential leucocytes counts in blood and sputum. Exercise induces a 2.2 fold increase in airway neutrophils (p=0.019) and 1.4 fold increase in blood neutrophils (p=0.01).

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	Neutrophils	Eosinophils	Lymphocytes	Monocytes
Sputum pre-exercise	2.47±0.8	0.06 ± 0.04	0.03 ± 0.01	0.35±0.1
Sputum post-exercise	5.5 ± 1.8	0.37±0.27	0.06 ± 0.02	0.77 ± 0.2
Blood pre-exercise	5.8 ± 0.8	0.13 ± 0.04	1.65 ± 0.1	0.5 ± 0.03
Blood post-exercise	$7.6 {\pm} 0.9$	$0.13{\pm}0.04$	$1.54{\pm}0.15$	$0.5 {\pm} 0.03$

Conclusion: We demonstrate that acute exercise increases the peripheral and air-

way inflammation in stable CF subjects. This burst of neutrophils might have a dual role in which it might improve local defense against infections and participate in airway destruction through release of elastase and other mediators. The modulation of this inflammatory response by exercise training has to be explored.

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Heart rate variability response to submaximal exercise in children with cystic fibrosis

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Background: Rehabilitation or physical activity can improve chronic respiratory disease as a Cystic Fibrosis. Nevertheless, because autonomic dysfunction is common in CF, heart control may be affected in response to exercise.

Objective: To assess the cardiac autonomic control using heart rate variability (HRV) analysis before and after a six-minute walking test (6MWT).

Methods: We studied lung function at baseline and HRV before and after 6MWT in children's with CF and matched healthy control group.

Results: Thirteen children in the CF group (6 male) mean age: 12 ± 2.7 years, with obstructive disease (FEV1/FVC: 0.83 ± 0.11 , FEV1: $71.4\pm21\%$ pred) and 12 healthy children (6 male), mean age 11.4 ± 2.4 years, with normal lung function (FEV1/FVC: 0.93 ± 0.12 , FEV1: $91.6\pm17.4\%$ pred) were evaluated. Baseline HRV was different between CF and CG in LFnu: 53.18 ± 15.01 vs. 32.79 ± 7.91 , p = 0.0003; HF%: 25.45 ± 18.43 vs. 53 ± 9.56 , p = 0.0018; HFnu: 47.32 ± 14.68 vs. 68.34 ± 8.67 , p<0.0019; and LF/HF: 1.25 ± 0.72 vs. 0.49 ± 0.18 , p < 0.0066. After the 6MWT was observed significant differences between groups for LF(m/s2): 846.69\pm754.81 vs. 345.58 ± 197.18 , p=0.027; LF%: 35.44 ± 8.06 vs. 25.88 ± 6.20 , p=0.0024; LFnu: 60.04 ± 16.27 vs. 39 ± 8.71 , p<<0.0001; HF%: 27.42 ± 13.73 vs. $48.13\pm6,33$, p=0.0003; HFnu: 40.45 ± 15.8 vs. 65.59 ± 8.18 , p=0.0003; and LF/HF: 1.9 ± 1.7 vs. 0.53 ± 0.21 , p=0.0001.

Conclusion: Children with CF had higher sympathetic drive at baseline and after a submaximal exercise test compared to the CG, suggesting a sympatho-vagal dysfunction.